

4-1949

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## Recommended Citation

Aronoff, S.; Haas, V. A.; and Fries, B., "Degradation of glucose-1-C14 and a possible new step in the mechanism of fermentation" (1949). *Ames Laboratory ISC Technical Reports*. 5.  
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# Degradation of glucose-1-C14 and a possible new step in the mechanism of fermentation

## **Abstract**

The availability of glucose-1-c14 has permitted the verification of a scheme of glucose degradation applied to sugars formed in photosynthesis to determine the distribution of isotopic carbon within the sugar. As a result of the present investigation, there appears to be a second, though minor, pathway of fermentation of the test organism, *Lactobacillus casei*.

## **Disciplines**

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UNITED STATES ATOMIC ENERGY COMMISSION

**DEGRADATION OF GLUCOSE-1-C<sup>14</sup> AND A POSSIBLE NEW STEP  
IN THE MECHANISM OF FERMENTATION**

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Date of Manuscript: April, 1949

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Technical Information Branch Oak Ridge, Tennessee  
AEC, Oak Ridge, Tenn., 5-23-49--97-A8385

# DEGRADATION OF GLUCOSE-1-C<sup>14</sup> AND A POSSIBLE NEW STEP IN THE MECHANISM OF FERMENTATION\*

By S. Aronoff, V. A. Haas, and B. Fries

The availability of glucose-1-C<sup>14</sup><sup>†</sup> has permitted the verification of a scheme of glucose degradation applied to sugars formed in photosynthesis to determine the distribution of isotopic carbon within the sugar (1). As a result of the present investigation, there appears to be a second, though minor, pathway of fermentation of the test organism, *Lactobacillus casei* C.

The degradation procedure is depicted in Table I and involves the following sequence of reactions: the fermentation of glucose to lactic acid; the oxidation of lactic acid to carbon dioxide (carbons three and four of the original glucose) and acetic acid; the pyrolysis of barium acetate to acetone and barium carbonate (carbons two and five); and the formation of iodoform (carbons one and six) from the acetone. The various steps in the process have been tested with (a) methyl- and carboxyl-labeled acetate, (b)  $\alpha$ -labeled lactate<sup>‡</sup>, and (c) glucose-1-C<sup>14</sup>.

Acetate has been pyrolyzed under a variety of conditions, a number of which can be so unfavorable (e.g. that at 450°C, with flowing argon), as to result in the appearance of more than eight percent of the activity of methyl-labeled acetate in the purified residual barium carbonate. Under similar conditions, with carboxyl-labeled acetate, less than one-half of one percent of the activity is in the iodoform. Using flowing argon, at a temperature of 530°C for ten minutes, and liberating the carbon dioxide from the residual carbonate with lactic acid, only 0.86 percent of the

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\*This research was initiated at the Radiation Laboratory, University of California, and continued at the Ames Laboratory, Iowa State College. The initial work was reported in UCRL-55.

<sup>†</sup>The glucose used by the senior author was synthesized by John C. Sowden, see, e. g., Science 109:229 (1949), "1-C<sup>14</sup>-D-Glucose and 1-C<sup>14</sup>-D-Mannose"; that used in California Laboratory was synthesized by H. Mahler according to Sowden's general method (UCRL-174, "The Synthesis of D-Glucose-1-C<sup>14</sup>").

<sup>‡</sup>The acetates were synthesized by Dr. B. Tolbert, and the lactic acid by Dr. R. Lemmon (both at the University of California Radiation Laboratory).





activity of the methyl-labeled acetate is found in the carbonate.

Electrolysis of sodium acetate (2), the products of which are carbon dioxide and ethane, resulted in one percent of the radio-activity of the methyl group in the carbon dioxide from methyl-labeled acetate.

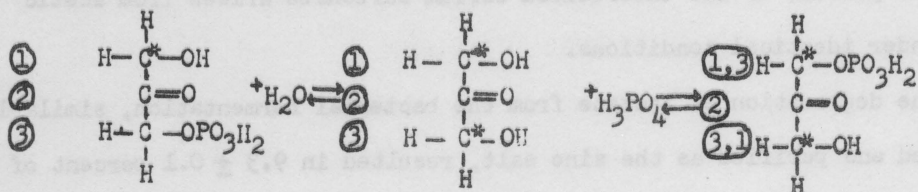
The chromium trioxide oxidation of  $\alpha$ -labeled lactate (3) purified and recrystallized as the zinc salt, resulted in 4.3 percent of the activity in the carbon dioxide evolved. This activity arises primarily from oxidation of compounds other than the acetic acid itself, as less than one percent of the theoretical barium carbonate arises from acetic acid under identical conditions.

The degradation of lactate from the bacterial fermentation, similarly isolated and purified as the zinc salt, resulted in  $9.3 \pm 0.1$  percent of the activity in the barium carbonate, rather than the empirical  $4.3 \pm 0.1$ . The difference of 5% must be ascribed to the activity of carbons three and four. In fermentation the carboxyl group of the lactic acid is presumed to arise solely from carbons three and four of glucose, whereas in ordinary chemical alkaline rearrangements resulting in lactic acid (4) the carboxyls may stem from carbons one and six. This is certainly so in the case of gluconic acid, where 1.3 moles of lactic acid per mole of gluconic acid can be obtained.

These experiments are in general accord with the recent note by Koshland and Westheimer (5) on the fermentation of glucose-1- $C^{14}$  by yeast. In yeast fermentation the carbon dioxide may be derived from a variety of intermediary products of metabolism. Furthermore the ability of yeast to fix carbon dioxide is pronounced. In view of these and the rather large counting deviations in their experiments, a quantitative interpretation of

their results is not possible.

At least three apparent explanations of our results are suggested, the latter two of which represent deviations from the normal fermentation mechanism: (a) that metabolic carbon dioxide (from the radioglucose) is reversibly reincorporated in lactic acid, in which case the activity would reside primarily in the carboxyl group, (b) that there is a fermentation mechanism in which carbons one and six rather than three and four are oxidized, (c) during the course of the normal fermentation mechanism the triose phosphates are in (partial) equilibrium with the free trioses, viz.



This would, of course, result in activity appearing in both carbons one and three of lactic acid to an extent depending on the amount of approach to an equilibrium. We have not investigated the enzyme systems involved and are thus not able to choose between the second and third explanations. The first explanation appears difficult to reconcile with the fact that the fermentation is conducted in M/10 bicarbonate (pH 6.8), with subsequent dilution of carbon dioxide formed by metabolism. Furthermore, control experiments with inactive glucose but with radio-carbonate ( $2.8 \times 10^6$  disintegrations per minute in 5 ml of reaction mixture) resulted in approximately 0.02 percent of the activity in the lactic acid formed. There still remains the possibility, albeit dubious, that the metabolic carbon dioxide is preferentially used at the site of formation, prior to the diffusion and subsequent dilution effects of the inactive bicarbonate of the solution.

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